had a majority of ARGS fragments (70, 61 and 77%, respectively), while the reference and the JIA groups had high proportion of CS2-G3 fragments (53 and 52%, respectively). Estimates of the relative mol-mol proportions of the pathological aggrecanase cleavage in the IGD generating ARGS-fragment and the aggrecanase turnover cut in the CS2 region generating CS2-G3 fragments showed that the ARGS proportion was 27% for the reference group while it was between 69 and 82% in the adult and juvenile injury groups and in OA group. A similar comparison of MMP generated FFGV (a cut in the IGD of aggrecan) and the aggrecanase generated ARGS fragments showed that the FFGV fragments amounted to 32% for the reference group while the proportion was much lower for the adult injury (2.1%), juvenile injury (7.3%) and the OA (1.2%) groups.

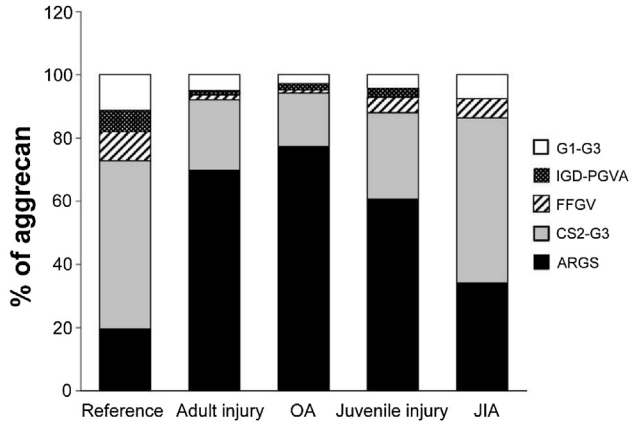

Fig 1. Proportion (mol/mol) of full length aggrecan (G1-G3), aggrecanase generated ARGS and CS2-G3 fragments, MMP generated FFGV fragments and calpain generated IGD-PGVA fragments found in synovial fluid pools of different patient Groups.

Conclusions: The OA, juvenile and adult knee injury groups show similar aggrecan fragmentation patterns, which differ from the fragmentation pattern of JIA and knee healthy reference groups. This suggests that the aggrecan fragmentation patterns are different between different joint diseases. This information supports further understanding of mechanisms of cartilage damage in these conditions, and may aid to distinguish different patient groups.

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RELATIONSHIP BETWEEN CONCOMITANT INJURIES SUSTAINED DURING ACL RUPTURE AND BIOLOGICAL MARKERS OF ARTICULAR CARTILAGE METABOLISM

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Purpose: There is limited information regarding the onset and earliest stages of post-traumatic osteoarthritis (OA), which is commonly associated with rupture of the anterior cruciate ligament (ACL). Consequently, the purpose of this investigation was to examine relationships between concomitant injuries to the tibiofemoral articular cartilage sustained during acute ACL injury with patient-oriented outcomes as well as biochemical markers of type II collagen metabolism and aggrecan degradation, compared to healthy, matched controls.
Methods: Thirty-nine ACL-reconstructed (20 women) and 32 knee healthy control (18 women) subjects matched for age, sex, race, BMI, and activity level were evaluated in this cross-sectional study. Inclusion criteria for injured subjects was: age 14-55yrs, BMI between 18.5-30, Tegner activity score $\geq$ 5, no previous knee pathologies, normal anatomic tibiofemoral alignment, $<$ 2/3 meniscectomy performed at surgery, and $\leq$ grade 3A articular cartilage lesions (based on International Cartilage Repair Society [ICRS] classification). Similar inclusion criteria were utilized for controls with the exception of: no history of knee pain or dysfunction, normal clinical knee examination, and no abnormalities on MRI. Articular cartilage lesions were identified under direct arthroscopic visualization at the time of ACL reconstruction and were documented by one of two sports medicine fellowship-trained orthopaedic surgeons. Injured subjects were classified as low-risk for future OA development if they displayed $\leq$ grade 2 articular cartilage lesions. Injured subjects were classified as high-risk for future OA development if they displayed grade 3A articular cartilage lesions. Synovial fluid samples were obtained from injured subjects immediately prior to surgery, and from controls at a single time point. The mean interval between index injury and surgery date was 70.1 days; range: 18-155 days. Synovial fluid markers of type II collagen synthesis were evaluated by measuring concentrations of procollagen II C-propeptide (CPII) with ELISA (Ibex). Markers of type II collagen degradation were also evaluated with ELISA and included collagen type II cleavage product (C2C; Ibex) and collagen type I and II cleavage product (C1,2C; Ibex). Additionally, the Alanine-Arginine-Glycine-Serine (ARGS) neoepitope was measured as a marker of aggrecan degradation using an electrochemiluminescence in-house immunoassay. Patient oriented outcomes were evaluated in all subjects with the Knee Injury and Osteoarthritis Outcome Score (KOOS). Analysis of Variance was performed for statistical evaluation.
Results: Of the 39 ACL reconstructed individuals, 29 (74%) had articular cartilage injuries that were grade 2 or less, while 10 (26%) had grade 3A articular cartilage injuries. Controlling for sex, BMI, activity level, and time between injury and baseline measurements, there were no significant differences in mean levels of markers of type II collagen metabolism or aggrecan breakdown ($p = 0.48$ and $p = 0.55$, respectively) between risk groups. Associations between ARGS concentration and KOOS subscales of symptoms and pain were found to be significantly different between groups ($p = 0.03$ and $p = 0.01$, respectively). These significant interactions were driven by positively correlated associations between KOOS scores and ARGS concentration for the high risk group, and negatively correlated associations between KOOS scores and ARGS concentration for the low risk group.
Conclusions: In ACL injured subjects with concomitant grade 3A articular cartilage injuries, levels of synovial fluid ARGS were directly associated with improvements in KOOS symptoms and pain. As a secondary analysis of a longitudinal investigation, this study provides preliminary, hypothesis generating data and may not be adequately powered to elucidate true differences in biomarker concentrations between these groups. Nevertheless, our statistically significant findings may suggest the involvement of synovial fluid ARGS in a localized tissue repair response involving an increase in the synthesis of aggrecan following traumatic knee injury.

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CARTILAGE COLLAGEN NEOEPITOPE C2C AND CLINICAL PARAMETERS IN MIDDLE-AGED PATIENTS WITH KNEE PROBLEMS. CORRELATIONS OF URINARY OUTPUT OF C2C WITH CARTILAGE LESIONS, KOOS VALUES AND FUNCTIONAL ABILITIES OF LOWER LIMB

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Purpose: Intensive research in the last decade has demonstrated that protein biomarkers are required for different purposes in early osteoarthritis (OA): to detect OA, to prognose its progression, to assess efficacy of intervention, etc. Although several biomarkers have acquired definite position in the field, none of the biomarkers applied up to now can sufficiently discriminate individual or limited number of patients (Labefer, van Spil, 2013). One of the likely ways to proceed is to investigate neoepitopes. A collagen type II neoepitope C2C was developed for this purpose.
The aims of the study were to test: (i) the biomarker's ability to differentiate between patients with and without knee cartilage lesion, (ii) if there is any correlation between urinary C2C output and clinical status of patients with early knee osteoarthritis, (iii) preferable option to express results (ng/mmol of creatinine or pg/ml of urine).
Material and methods: We investigated 180 knee OA patients (68 male, 112 female) aged 36-62 (mean 50) yrs. For 112 patients the progression of the knee OA during the past 3 years was available. Standardised radiographs of the tibiofemoral(TF) and patello[[Unsupported Character – Codename -]]femoral(PF) joints were assessed. Radiographic progression was defined as: (i)presence of osteophytes and/or joint space narrowing (JSN) in subjects with no previous radiographic OA or (ii)increase in their grade.